

AMENDMENT UNDER 37 C.F.R. § 1.111
Application No. 09/582,842

2. (Amended) The biotin-avidin-biotin complex according to claim 1, wherein at least one of said biotinylated substances is a biotinylated binding component and at least one of said biotinylated substances is a biotinylated labeling substance.

3. (Amended) A process for preparing said biotin-avidin-biotin complex according to claim 1, comprising the steps of:

- Q1*
- (1) treating an avidin with a crosslinking agent to prepare a crosslinked avidin;
 - (2) biotinylating the same or different substances to be biotinylated to prepare the same or different biotinylated substances; and
 - (3) binding said crosslinked avidin and said same or different biotinylated substances to form said biotin-avidin-biotin complex according to claim 1.
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6. (Amended) A method for analyzing a compound to be analyzed, said method comprising the steps of:

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- (1) providing a sample suspected of containing said compound to be analyzed;
 - (2) bringing into contact sequentially and in any order said sample, a biotinylated binding component capable of specifically binding said compound, a crosslinked avidin, and a biotinylated labeling substance, to form a complex of said compound to be analyzed, said biotinylated binding component, said crosslinked avidin, and said biotinylated labeling substance; and
 - (3) analyzing a signal derived from said labeling substance in said complex.

7. (Amended) The analyzing method according to claim 6, wherein said binding compound is selected from the group consisting of an antibody, an antibody fragment, an

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antigen, a DNA, an RNA, a receptor, a ligand to a receptor, an enzyme, a ligand to an enzyme, an enzyme analogue, a substrate for an enzyme which is an origin of an enzyme analogue, a lectin, and a sugar.

9. (Amended) The analyzing method according to any one of claims 6 to 8, wherein said biotinylated labeling substance is selected from the group consisting of a biotinylated enzyme, a biotinylated fluorescent substance, a protein bound to a biotinylated fluorescent substance, a biotinylated luminescent substance, a protein bound to a biotinylated luminescent substance, and a biotinylated radioactive isotope.

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10. ~~(Amended)~~ The analyzing method according to claim 9, wherein said biotinylated enzyme is a biotinylated fused ~~protein~~ of an enzyme and a biotin acceptor.

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11. (Amended) The analyzing method according to claim 9, wherein said biotinylated enzyme is a biotinylated luciferase.

12. (Amended) The analyzing method according to any one of claims 6 to 8, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptavidin, and a crosslinked recombinant avidin.--

Please add the following new claims:

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--24. (New) The analyzing method according to claim 9, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptavidin, and a crosslinked recombinant avidin.

25. (New) The analyzing method according to claim 10, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptoavidin, and a crosslinked recombinant avidin.

26. (New) The analyzing method according to claim 11, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptoavidin, and a crosslinked recombinant avidin.

27. (New) An analyzing reagent comprising a mixture of:

- (1) a biotinylated binding component;
- (2) a crosslinked avidin; and
- (3) a biotinylated labeling substance.

28. (New) The analyzing reagent of claim 27, wherein said binding component is selected from the group consisting of an antibody, an antibody fragment, an antigen, a DNA, an RNA, a receptor, a ligand to a receptor, an enzyme, a ligand to an enzyme, an enzyme analogue, a substrate for an enzyme which is an origin of an enzyme analogue, a lectin, and a sugar.

29. (New) The analyzing reagent of claim 28, wherein said antibody fragment is an Fab' fragment.

af 30. (New) A method for analyzing a compound to be analyzed, said method comprising the steps of:

- (1) providing a sample suspected of containing said compound to be analyzed;
- (2) providing a biotin-avidin-biotin complex comprising a biotinylated binding component and a biotinylated labeling substance, and a crosslinked avidin sandwiched therebetween;

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(3) bringing said sample into contact with said biotin-avidin-biotin complex to form a complex
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conceded* of said compound to be analyzed and said biotin-avidin-biotin complex; and

(4) analyzing a signal derived from said labeling substance in said complex formed in step (3).--
